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Expression of HSP90 and HIF-1 α in human colorectal cancer tissue and its significance

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ABSTRACT

Objective: To investigate the expression of HSP90 and HIF-1 α in human colorectal cancer tissue, the influence of HSP90 and HIF-1 α on human colorectal cancer biological behavior and their related factors. **Methods:** The expression of HSP90 and HIF-1 α protein in human colorectal cancer as well as normal tissue were detected by immunohistochemical method. **Results:** The positive expression rates of HSP90 and HIF-1 α protein in normal human colorectal tissue as well as colorectal cancer tissue were 30% vs. 63.0%, 15.0% vs. 71.7%, respectively. There were significant difference ($P=0.035$ and $P=0.005$ respectively). The expression of HSP90 was significantly correlated with the differentiation, Dukes stages and lymph node metastasis ($P<0.05$), while the expression of HIF-1 α was significantly correlated with the Dukes stages and lymph node metastasis ($P<0.05$). Association analysis showed that the expression of HSP90 protein was significantly correlated with that of HIF-1 α protein ($P<0.01$). **Conclusions:** The expression of HSP90 and HIF-1 α protein may be related to the development, metastasis and invasion of human colorectal cancer; and their synergistic effects may participate in the development of the colorectal carcinoma.

1. Introduction

Colorectal cancer includes large bowel cancer (colon cancer) and cancer of the back passage (rectal cancer or cancer of the rectum), with higher morbidity and mortality. In recent years, its prevalence shows an upward trend year by year and the ratio of male to female is about 1:1[1]. Heat shock protein 90 (Hsp90) plays an important role in vascularization, invasion and metastasis of tumors, proliferation of tumor cells, time course of cell cycles, as well as the conformation, stability and function maintenance of several carcinogenic proteins involving in

the signal transduction pathway of cell apoptosis[2]; while Hypoxia-inducible factor (HIF)-1 α could regulate tumor angiogenesis, glycolysis and invasiveness of tumors[3–5]. In our study, we detected the expression levels of HSP90 and HIF-1 α by immunohistochemical SABC method in the colorectal cancer tissues, observed their relationships with the clinicopathologic features of colorectal cancer and explored their correlations, in order to investigate the effects of HSP90 and HIF-1 α on the occurrence and development of colorectal cancer, as well as their roles in the invasion and metastasis process.

2. Materials and methods

2.1. General data

Paraffin-embedded tissue samples between October 2012

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and January 2004 and the fresh colorectal cancer tissues by surgical resection between October 2012 and January 2013 were selected. None had radio- and chemotherapy before and all cases were verified by pathological examination after operation. 46 cases of colorectal adenocarcinoma were enrolled in total, including 28 male and 18 female patients. The median age was 60.5 (36–81) years. These 46 cases included 25 cases with colon cancer and 21 cases with rectal cancer; 29 cases with high and medium differentiated adenocarcinoma and 17 cases with poorly differentiated adenocarcinoma; 16 cases with lymphatic metastasis and 30 cases without lymphatic metastasis; 30 cases at Dukes A+B and 16 case at Dukes C+D in accordance with the Dukes' stage. Meanwhile, 20 normal tissues were selected more than 10 cm away from the site of the tumor as the control group.

2.2. Primary reagents and methods

Mouse anti-human HSP90 monoclonal antibody (at a dilution of 1:100) and mouse anti-human HIF-1 α monoclonal antibody (1:100) were purchased from Abcam. Secondary and third antibodies were obtained from Vector and Sigma respectively.

They were fixed by 4% paraformaldehyde, dehydrated, transparentized and paraffin embedded to obtain 4 μ m serial paraffin sections. Then slices were baked using an electrothermostat, xylene dewaxed to water and membrane rupture. Slices were heated by two changes of medium temperature, heat-induced microwave in solution of citrate buffer (pH 6.0) for 5 min, with pause duration for 3 min. Thereafter, all the slices were washed with PBS and added with 3% H_2O_2 to initiate the reaction away from light at room temperature for 30 min. They were washed again with PBS and the reaction started in the incubator at 37 $^{\circ}C$ for 20 min with the diluted enzyme k (1:2 000). They were washed again, reacted in the incubator at 37 $^{\circ}C$ for 30 min with 2% BSA; the slices were dried as much as possible to remove the liquid, then primary antibody was added. They were incubated for 36 h at 4 $^{\circ}C$ and equilibrated to room temperature for 2 h. They were washed again, added with secondary antibody and incubated at 37 $^{\circ}C$ for 1 h. Then they were washed with PBS, added with SABC solution to co-incubate at 37 $^{\circ}C$ for 1 h. Remaining antibody was washed off, then they were developed with DAB for 8–10 min, redyed with hematoxylin, then the slides were dehydrated routinely until they became transparent. They were sealed with neutral resin. In negative control group the primary antibody was replaced with PBS, while in the positive control group the available HSP90 and HIF-1 α positive colorectal cancer tissue slices were used.

2.3. Evaluation criteria of the results

5 high-power fields were selected randomly in each slice, the percentages of positive tumor cells were: <10%, 0; 10%–25%, 1; 26%–50%, 2; 51%–75%, 3; >75%, 4. Staining intensity scores were: 0 was into colorless, 1 was divided into weak (pale yellow), 2 was divided into medium (brown), 3 was classified as strong (tan). Finally, the percentages of positive tumor cells were multiplied by the staining intensity scores: ≤ 2 was divided into negative and ≥ 3 was divided into positive.

2.4. Statistical analysis

SPSS 12.0 software was used for statistical analysis of all the results, in which Spearman correlation test was adopted for correlation analysis and *Chi*-square test for data comparison between both groups. $P < 0.05$ was considered as statistical significant difference.

3. Results

3.1. Expression percentages of HSP90 and HIF-1 α in colorectal cancer tissues and normal tissues

HSP90 was mainly expressed in the cytoplasm and nuclei of tumor cells as brown particles (Figure 1A), with the positive percentages in the normal tissues were significantly lower than those in the colorectal cancer tissues (30.0% vs. 63.0%) ($\chi^2 = 6.110$, $P = 0.013$) (Table 1). The amount of HIF-1 α expressed in the cytoplasm and/or nuclei was highest, and appeared as brown particles (Figure 1B). The positive percentages of HIF-1 α in the colorectal cancer tissues were 15.0% and 71.7% respectively, with statistical significant difference ($\chi^2 = 18.100$, $P < 0.001$) (Table 1).

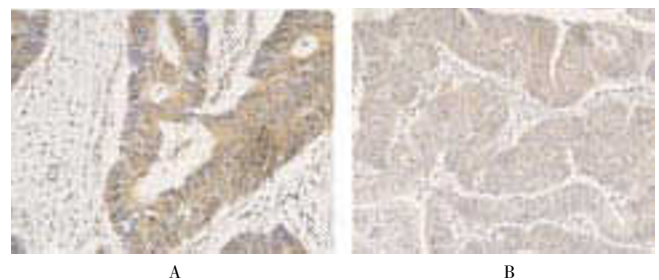


Figure 1. Expressions in colorectal cancer tissues (SABC method, magnification 400 times). A: HSP90; B: HIF-1 α .

Table 1

Expression percentages of HSP90 in colorectal cancer tissues and normal tissues.

Group	n	HSP90		HIF-1 α	
		+	–	+	–
Normal tissues	20	6	3	17	14
Colorectal tissues	46	29	33	13	17

3.2. Expressions of HSP90 and HIF-1 α in colorectal cancer and their respective correlations with the clinicopathologic features of colorectal cancer

There was no correlation of HSP90 expressions with patients' sex, age, site of tumor without statistical significances ($P>0.05$). We found that HSP90 expressions were related with tumor differentiated degree, Dukes staging and lymph nodes metastasis with statistical significances ($P<0.05$), which were in line with the results from Dong Xing *et al*[6–8]. Also, there was no correlations of HIF-1 α expressions with patients' sex, age, site of tumor without statistical significances ($P>0.05$). We found that HSP90 expressions were related with Dukes staging and lymph nodes metastasis with statistical significances ($P<0.05$), which coincided with the results from Yoshimura *et al*[1,9] (Table 2)

Table 2

Expressions of HSP90 and HIF-1 α in colorectal cancer and their respective correlations with the clinicopathologic features of colorectal cancer.

Clinicopathologic features		n	HSP90			HIF-1 α		
			Positive (%)	χ^2	P	Positive (%)	χ^2	P
Sex	Male	28	68.5	0.047	0.828	67.9	0.532	0.466
	Female	18	61.1			77.8		
Age	≤ 60	22	68.2	0.478	0.489	68.2	0.263	0.608
	> 60	24	58.3			75.0		
Site of tumors	Colon	25	56.0	1.166	0.280	72.0	0.002	0.966
	Rectum	21	71.4			71.4		
Differentiation degree	High and medium differentiated adenocarcinoma	29	51.7	4.315	0.038*	79.3	1.323	0.250
	poorly differentiated adenocarcinoma	17	82.4			58.8		
DUKES staging	Stage A+B	30	50.0	6.298	0.012*	60.0	4.316	0.038*
	Stage C+D	16	87.5			93.8		
Lymph nodes metastasis	Yes	30	87.5	6.298	0.012*	93.8	4.316	0.038*
	No	16	50.0			60.0		

3.3. Correlation of HSP90 and HIF-1 α expressions in colorectal cancer tissues

There were 25 cases with positive HSP90 and HIF-1 α , 9 cases with negative HSP90 and HIF-1 α . Eight positive HIF-1 α cases showed negative HSP90 result; while 9 positive HSP90 cases showed negative HIF-1 α result. Spearman correlation test showed that a positive correlation between HSP90 and HIF-1 α in colorectal cancer tissues ($\gamma=0.420$, $P<0.01$).

4. Discussion

The occurrence, development, metastasis and invasion of neoplasms are result from interactions among polygenes, multiple factors and multi-stages.

HSPs, also known as molecular chaperon is essential for regulating intracellular protein balance with highly conserved amino acid sequence. Its main functions include direct protein folding within the cytoplasm, endoplasmic reticulum and mitochondria and its transmission and intracellular localization, repair and degrade denatured proteins and refold the misfolded proteins to prevent misparing and accumulation of proteome. In addition, HSPs

involves in the final step of activations of several regulatory proteins in eukaryotic cells, the remodeling of several macromolecular protein complexes which comprises the signal transduction, transcription, cell division and migrated and differentiated proteins. Furthermore, in the presence of stressors such as high temperature, heavy metal, hypoxia and acidosis, the content of HSPs will increase^[10,11].

Based on the molecular weight of HSPs, it can be classed into several types such as HSP110, 90 and 70^[12]. HSP90 which is mainly comprised of HSP90a and HSP90 β , is a macromolecular protein with the molecular weight of 90KD, largest part of which exists in GRP94 of the endoplasmic reticulum (glucose regulated protein 94) and TRAP1 of the mitochondria (TNF receptor regulating protein)^[13]. Under physiological status, HSP90 accounts for 1% to 2% total proteins in cells; while in a state of stress, its content increased by about 2 to 10 times^[6,10,11]. It has been demonstrated in previous studies that, the expression levels of HSPs in several solid tumors (breast cancer^[10], hepatocellular carcinoma^[14], gastric cancer, nasopharynx cancer and ovarian cancer^[15,16]) and hematological malignancies have been increased^[10].

The target proteins of HSP90 include matrix metalloproteinase 2 and urokinase involving in the process of tumor cells invasion and metastasis, hypoxia-inducible factor (HIF) that maintains revascularization, epidermal growth factor receptor (rEGFR), steroid receptor coactivator and *etc*^[11,13], which play a role by regulating the function of protein kinase B, tumor necrosis factor receptor, nuclear factor κ B to inhibit the apoptosis of tumor cells. Researches have shown that the expression levels of HSP complex in colon cancer are correlated with proliferating cell nuclear antigen, considering HSP complex may contributes to the rapid proliferation of tumor cells^[7,11]. In our study, the positive percentages of HSP90 in normal and colorectal adenocarcinoma tissues were 30% and 63%, respectively, with statistical significance. Therefore, the apparently higher expression levels of HSP90 in colorectal cancer tissues than those in the normal tissues indicates that the existing stressors such as hypoxia and acidosis at the beginning of colorectal cancer formation may contribute to the statistically higher HSP90 expression level in colorectal cancer tissues than those in the normal tissues. Meanwhile, it is demonstrated that there are statistical differences between both groups in the expressions and differentiation degree of HSP90, Dukes staging and their relationship with lymph nodes metastasis ($P<0.05$), which indicate that HSP90

not only forms at the early stage of colorectal cancer, but also involves in several processes such as differentiation, invasion and metastasis.

HIF-1, a transcriptional activator of basic helix-loop-helix/PER-ARNT-SIM, is comprised of HIF-1 β and inducible expressed HIF-1 α . The continuous expression of HIF-1 β is not effected by the environment. As the major regulating component, the content of HIF-1 α may be increased by several approaches like hypoxia and genetic variation^[4,17–19]. Higher expressions of several types of tumors like colon, breast, gastric, lung, skin, ovarian, pancreatic, prostate and renal carcinoma can be observed^[20].

As a transcriptional activator, HIF-1 α involves in the gene coding procedures of 13 different glucose transporters, glycolytic enzymes and VEGF, as well as the coding of erythropoietin, transferrin, endothelin-1, inducible synthetase, hemeoxygenase 1, IGF-2, IGF binding protein 2 and 3, where most of the proteins are related to the occurrence and development of tumors^[3,20]. In our study, the positive percentages of HIF-1 α in normal and colorectal cancer tissues are 15.0% and 71.7%, respectively with statistical significance ($P<0.05$). Along with previous study observations that high expression levels of HIF-1 α in noncancerous lesions like colonic adenoma and prostatic intraepithelial neoplasia^[20–22], it can be manifested that the increased levels of HIF-1 α expression may be the results of the higher expressions of several genes that facilitate the occurrence and development of tumors, thus provide advantageous environment for the occurrence of colorectal cancer. In addition, HIF-1 α can regulate and encode genes that play an important role in the pathophysiological process of cell invasion which including cathepsin D, MMP2, urokinase-type plasminogen activator receptor, fibronectin 1, vimentin TGF α , autocrine motility factors, keratin 14, 18 and 19. It has been verified that even if tissues experience an instant of hypoxia, the invasiveness of tumor cells towards basilar membrane *in vitro* also increase. While in our study, the expression of HIF-1 α correlates with Dukes staging and lymph nodes metastasis with statistical significance ($P<0.05$), indicating HIF-1 α may also participate in the gene regulation of proteins related to tumor metastasis and invasion and is essential for the invasion and metastasis of colorectal cancer.

According to the analysis of our results, there is a positive correlation between HSP90 and HIF-1 α ($r=0.420$), with statistical significance ($P<0.01$), showing they may exert a synergistic effect on the occurrence, development, invasion

and metastasis of colorectal cancer. Therefore, it reminds us the combination of HSP90 and HIF-1 α inhibitor may further improve the treatment efficacy of colorectal cancer.

Conflict of interest statement

We declare that we have no conflict of interest.

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